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Release of carbon in different molecule size fractions from decomposing boreal mor and peat as affected by Enchytraeid worms

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Abstract

Terrestrial export of dissolved organic carbon (DOC) to watercourses has increased in boreal zone. Effect of decomposing material and soil food webs on the release rate and quality of DOC are poorly known. We quantified carbon (C) release in CO_2, and DOC in different molecular weights from the most common organic soils in boreal zone; and explored the effect of soil type and enchytraeid worms on the release rates. Two types of mor and four types of peat were incubated in laboratory with and without enchytraeid worms for 154 days at +15°C. Carbon was mostly released as CO_2; DOC contributed to 2-9 % of C release. The share of DOC was higher in peat than in mor. The release rate of CO_2 was three times higher in mor than in highly decomposed peat. Enchytraeids enhanced the release of CO_2 by 31-43 % and of DOC by 46-77 % in mor. High molecular weight fraction dominated the DOC release. Upscaling the laboratory results into catchment level allowed us to conclude that peatlands are the main source of DOC, low molecular weight DOC originates close to watercourse, and that enchytraeids substantially influence DOC leaching to watercourse and ultimately to aquatic CO_2 emissions.

Key words: carbon dioxide, dissolved organic carbon, enchytraeids, organic matter, peat, mor

47 1. Introduction

48

49 Under aerobic conditions, decomposition of organic matter produces carbon dioxide (CO_2)
50 into the atmosphere and clearly less dissolved organic carbon (DOC) into the soil solution. In
51 boreal mor and peat saprophytic fungi are typically the primary decomposers of organic
52 matter. Soil fauna, especially fungivores, play a key role in enhancing soil nutrient cycling
53 and site productivity (Huhta et al. 1998, Popatov and Tiunov 2016) in boreal forests, and
54 their function is reflected to carbon (C) release as well. Soil fauna enhance the release of C
55 by fragmenting organic matter into smaller particles and by grazing upon microbes, thereby
56 stimulating mineralisation and increasing the solubility of organic C (Briones et al. 1998;
57 Bardgett and Chan 1999; Laakso and Setälä 1999). Functionally, the most influential faunal
58 group in boreal upland and peatland forest soils is the enchytraeid worms (Laakso and Setälä
59 1999; Silvan et al. 2000), of which more than 95 % comprise but one species, *Cognettia*
60 *sphagnetorum* (Vejdovsky) (Nurminen 1967; Abrahamsen 1972).

61

62 In the ecosystem C balance, DOC export links the terrestrial and aquatic systems together
63 (Huotari et al. 2011) as water eventually transports the terrestrial DOC into water courses. In
64 boreal lakes the majority of the lake DOC is allocthonic, i.e. originates from the terrestrial
65 part of the catchment (Jonsson et al. 2001; Porcal et al. 2009). DOC export is affected by
66 hydrology, catchment characteristics, and land-use (Sarkkola et al. 2009; Palviainen et al.
67 2016), but the controlling factors behind DOC quality are still unclear. The share of DOC
68 that reaches the water course depends on the transport time and degradability of DOC. The
69 labile fraction (half-life days or weeks) is likely degraded in the soil during the transport
70 process, and therefore will be detected as terrestrial CO_2 efflux, whereas the refractory (half-
71 life years) DOC more likely ends up to water course and is finally emitted as aquatic CO_2
72 efflux.

73

74 The degradability of DOC is related to the origin of the organic matter and the
75 decomposition stage of DOC (Kiikkilä et al. 2014; Mastný et al. 2018). Fresh litter and root
76 exudates are the main source of labile DOC in soil (Yano et al. 2000; Kalbitz et al. 2003a;
77 Kiikkilä et al. 2006), and refractory DOC compounds typically originate from highly
78 decomposed organic matter and microbial metabolites (Kalbitz et al. 2003b). During
79 decomposition, DOC becomes enriched in aromatic structures and therefore becomes higher
80 in molecular weight (Kalbitz et al. 2003b; Hagedorn and Machwitz 2007). Thus low
81 molecular-weight DOC (LMW-DOC) is often considered mostly labile, whereas high
82 molecular-weight DOC (HMW-DOC) is considered more refractory (Marschner and Kalbitz
83 2003). The proportion of labile fraction in soil DOC pool varies considerably (e.g., Kalbitz et
84 al. 2003a; van Hees et al. 2005; Kiikkilä et al. 2006). The size of the labile pool can change
85 rapidly due to its fast turnover (Kiikkilä et al. 2014), as well as due to physico-chemical
86 sorption and complexes forming processes, which retain labile DOC in the organic soil
87 matrix (Müller et al. 2009). The labile and refractory compounds are interacting as the
88 presence of easily degradable DOC can enhance the decomposition of more refractory
89 compounds (Lindén et al. 2014, Liu et al. 2017). Also soil fauna enhances biodegradation of
90 refractory C pool (Fox et al. 2006; Briones et al. 2007), but the contribution of soil fauna to
91 the release rate of DOC in labile and refractory fractions remains poorly known.
92
93 Quantification of the decomposition products in labile and refractory fractions is a
94 prerequisite for understanding the mechanisms of DOC export to watercourses and for
95 developing process-based solute transport models. Laurén et al. (2012) conducted a
96 laboratory experiment to obtain these data for further development of the decomposition
97 model ROMUL (Chertov et al. 2001) with special emphasis on solute transport applications.
98 Based on the study of Laurén et al. (2012) an extension of the ROMUL was presented to
99 simulate the dynamics of DOC for a single mor humus type (Laine-Kaulio et al. 2014), but
100 extension and application of the model to catchment scale need additional knowledge about
101 the release rates of CO_2 and DOC in different molecule sizes across a wider range of organic

soil types. These needs, i.e. requirement of the model parameterisation and validation, are addressed in the current study, where we aim at quantifying C release rates from the most common organic soil types in boreal coniferous forests.

Our objective was to explore the effect of soil type and the role of presence/absence of enchytraeids on the rate of C release. We applied a controlled experimental setup, where we incubated organic soil samples in constant temperature and soil moisture, and we used the LMW and HMW fractions of DOC as estimates for labile and refractory DOC.

We incubated mor, slightly decomposed peat, and highly decomposed peat, two types of each, and hypothesised that 1) the differences among the decomposing materials are reflected in the release rates of CO_2-C, HMW-DOC and LMW-DOC, and that 2) the presence of enchytraeid worms enhances the release of both DOC and CO_2. Because *Sphagnum* residues typically decompose slowly (e.g., Johnson and Damman 1993), we expected the release rates of C to be lower in peat than in mor. Furthermore, we expected slightly decomposed peat to release more C than highly decomposed peat. Finally, on account of the more preferential food resources and moisture conditions (Didden 1993; Silvan et al. 2000), we expected enchytraeids to be more influential in mor than in peat and more influential in slightly decomposed peat than in highly decomposed peat. Using the obtained results, literature and simple computation we quantitatively discuss how our results can reflect to DOC export to water courses typical boreal catchment.

2. Material and methods

2.1 Study sites and sampling

Soil samples representing the most common organic soils in boreal region were collected from Sotkamo, eastern Finland. Six soil types were included in the study: i) medium fertility

130 mor from Mesic forests, and ii) low fertility mor typical to Sub-xeric forests (Tomppo
131 2000), iii) slightly decomposed *Carex*-*Sphagnum* peat, iv) highly decomposed *Carex*-
132 *Sphagnum* peat, v) slightly decomposed *Sphagnum*-peat, and vi) highly decomposed
133 *Sphagnum*-peat. Mesic and Sub-xeric forests comprise 78 % of the upland forests in Finland
134 (Finnish Statistical Yearbook of Forestry, 2014), and *Sphagnum*-dominated peat represents
135 49 % and *Carex*-dominated peat 37 % of the peatlands in Finland (Virtanen et al. 2003).
136
137 Long-term (1981-2010) mean annual precipitation in the area was 591 mm, with about 40 %
138 falling as snow, and the mean annual air temperature was +2.3 °C (Pirinen et al. 2012), the
139 mean monthly temperature ranges from -10.7 °C (January) to 16.4 °C (July). The annual
140 mean temperature at the top most soil layer varies from 3.8 °C to 4.9 °C (Palviainen et al.
141 2004), the depth of snow cover from 72 cm to 92 cm, and maximum depth of soil frost from
142 3 to 24 cm (Finér et al. 1997).
143
144 Mor samples were collected from Kangasvaara and Kangaslampi catchments (63°51'N
145 /28°58'E, altitude 220 m above mean sea level, Finér et al. 1997). Forest site types were
146 classified as the *Vaccinium myrtillus* type in Kangasvaara and as the *Empetrum vaccinium*
147 type in Kangaslampi (Cajander 1949). The forest was old-growth Norway spruce (*Picea*
148 *abies* (L.) Karsten) mixed mainly with Scots pine (*Pinus sylvestris* L.). In Kangasvaara the
149 thickness of the organic layer ranged from 5 to 9 cm and in Kangaslampi, from 3 to 5 cm.
150
151 Peat samples were collected from drained pine bogs from Koivupuro and Suopuro (63°52'N
152 /28°39'E, altitude 200 m above mean sea level, Ahtiainen and Huttunen, 1999) catchments.
153 In Koivupuro the peat layer depth was 1-5 m and in Suopuro, 1.5-2 m. The dominant tree
154 species in the catchments was Scots pine, and the ground vegetation cover represented the
155 dwarf-shrub type (Cajander 1949).
156

157 The sample plot size was 50 m x 50 m, in which two parallel lines (25 m apart) were
158 established and four soil samples were collected with 10 m intervals in each line. Micro-site
159 for the sampling was a topographically even spot located at least 1 m away from a nearest
160 tree. A total of 48 soil samples were collected for the laboratory incubation, i.e. eight
161 laboratory replicates for each soil type. A cylindrical core (diameter 20 cm, height 9-20 cm)
162 was extracted from the organic layer. All living above-ground vegetation was carefully
163 removed from all the samples. Due to the thin organic layer in the low-fertility upland site,
164 the samples of this material were constructed of two to four layers placed into the container
165 layer by layer until the thickness of the sample was ca. 10 cm as was done in Laurén et al.
166 (2012). This construction provided a sufficient soil volume for soil solution sampling during
167 the incubation experiment. The procedure was considered to cause less soil disturbance than
168 sample construction by homogenisation and repacking of soil material. The peat samples
169 were collected from the surface layer (down to depth of ca. 20 cm) to represent slightly
170 decomposed peat (H3-H4 on the von Post (1922) scale of decomposition) and from the
171 underlying layer (down to depth of ca. 40 cm) to represent highly decomposed peat (H6-H7
172 on the von Post scale of decomposition). The incubation samples need to be undisturbed
173 throughout the experiment; therefore, we collected parallel samples for analyses requiring
174 destructive soil sampling (the basic soil characteristics and the extractable C contents).
175 Additional mor material was collected for extraction of enchytraeid worms, which were
176 subsequently inoculated into half of the soil containers as described below. Prior to the worm
177 extraction, the soil material was stored at +4 °C.
178
179
180 2.2 Incubation environment and soil analyses before the incubation
181
182 The interaction between the saprophytic fungi (here primary decomposers) and the
183 fungivores (here enchytraeid worms) becomes visible when we compare C dynamics
184 between samples including primary decomposers and fungivores against a system where

185 only the primary decomposers are present. Therefore, before the incubation the meso- and
186 macrofauna in the soil containers were killed by freezing the soil containers to a temperature
187 of -20 °C, after which the contents were allowed to thaw (Setälä et al. 1988). This procedure
188 was repeated twice. Freezing of soil is not particularly radical treatment because the upmost
189 organic layer freezes annually also in field conditions. The defaunation treatment and the
190 decaying roots in the soil samples, may have caused a momentary C flush, which was taken
191 into account in the calculation by omitting the first measurements and thereafter considering
192 the long-term rate of C release. The defaunation manipulated the faunal community in the
193 samples, but was not likely to change the primary decomposer communities, especially
194 fungi. Fungi can survive in temperatures far below -20 ℃ (e.g. Lehto et al. 2008; Kilpeläinen
195 et al. 2016) allowing us to assume that the original diverse microbial population was present
196 throughout the incubation experiment.
197
198 After the defaunation, the water content of the soil samples was adjusted to correspond to
199 field capacity by wetting the soil samples with deionised water to the point where water
200 started to seep through a hole in the bottom of the container. Surplus water was allowed to
201 drain through the hole until the seepage ceased. The hole was then closed, and the container
202 was weighed. The containers were placed in a dark growth chamber (GR77, Conviron
203 Controlled Environments Ltd., Canada) with a constant temperature of +15 °C and a relative
204 humidity of 80 % for the incubation period of 154 days. The incubation temperature was set
205 to typical summertime topsoil temperature in the study area (Palviainen et al. 2004). Half of
206 the containers, i.e., four for each soil type, were inoculated with enchytraeids extracted using
207 the wet funnel method by O'Connor (1962). About 50 worms per container were inoculated
208 at the beginning of the incubation, and a further 50 worms monthly to assure the continuous
209 presence of enchytraeids in the containers. The number of enchytraeids inoculated during the
210 experiment (per container) corresponded to a total of ca. 8 000 individuals m^{-2}, or 0.1 g m^{-2}
211 in terms of the dry mass of worms representing a typical *Cognettia* -population density in
212 field conditions in these kind of sites (Räty and Huhta 2004). The total number of

213 enchytraeids in the soil containers was determined at the end of the incubation allowing us to
214 evaluate the magnitude of reproduction during the experiment.
215
216 At the beginning of the experiment, the soil C/N, pH, and contents of extractable C
217 compounds were determined from the parallel samples. The total C and N contents were
218 determined using CHN analyser (CHN-2000, LECO Corporation, USA). Soil pH was
219 measured from a suspension of soil in H_2O (1:2 v:v). Extractable organic C (OC_{ex}) was
220 extracted with 0.5M K_2SO_4 and analysed using a total organic carbon analyser (TOC 5000A,
221 Shimadzu Scientific Instruments, Inc., USA). OC_{ex} is assumed to include both DOC in the
222 solution and the organic C adsorbed on solid surfaces. Microbial biomass C (C_{mic}) was
223 determined using the fumigation-extraction method (Sparling et al. 1990; Vance et al. 1987).
224 The extracts were filtered through 0.45 µm filter before the TOC analyses. Basic
225 characteristics of the soil types are reported in Table 1 and the initial pools of the C
226 compounds studied, in Table 2.
227
228 2.3 Sampling and analyses of the soil solutions during the incubation
229
230 During the incubation, soil solution samples were repeatedly collected using suction
231 samplers (MacroRhizon with syringe, Eijkelkamp, The Netherlands). The suction sampler
232 consisted of a polymeric porous tip (9 cm long and 4.5 mm in diameter) attached to a
233 removable syringe generating a suction of approximately -100 kPa. The mean pore size of
234 the sampler tip was 0.1 µm, which is slightly less than the widely used cut-off limit for DOC
235 filters (0.45 µm). The smaller pore size was preferred for this study as it enables soil solution
236 sampling also in rather dry conditions. Two sampling tips were inserted into each container
237 vertically. In each sampling event, ca. 100 ml of soil solution was collected, which took 2-3
238 days. The mass of each soil solution sample was determined before the analysis. The
239 sampling was repeated eight times at 2-6 week intervals, with the shortest intervals at the
240 beginning of the experiment. The water lost through evaporation and the soil solution

241 sampling was compensated once a week by adding deionised water into the containers until
242 their original mass was reached.
243
244 The soil solution samples were divided into two parts. One part was filtered (Amicon Stirred
245 Cell model 8400, Millipore Corporation, USA, pressure 1.5-2 bar) through an ultrafiltration
246 membrane with a nominal molecular weight limit of 1 kDa. In the filtration, two thirds of the
247 load volume was allowed to pass through the membrane. The ultrafiltered fraction represents
248 the low molecular-weight fraction of DOC (LMW-DOC). The other part of the original soil
249 solution sample remained unfiltered. DOC was determined from both the filtered and the
250 unfiltered samples (TOC-5000A). The relative molecular-size distributions of DOC and
251 LMW-DOC were determined using the size-exclusion chromatography analysis (HPLC,
252 Agilent Technologies, USA). The wavelength was set at 254 nm, and the injected sample
253 volumes varied between 5 and 30 µl, depending on the DOC concentration of the sample.
254 Seven different relative molecular-size classes were distinguished, based on the peaks in the
255 chromatography results, with the size decreasing from class 1 to class 7. The comparison of
256 size classes in the DOC and LMW-DOC samples revealed that classes 1 and 2 were missing
257 from the LMW-DOC fraction. Thus, classes 1 and 2 represented the high molecular-weight
258 DOC (HMW-DOC), i.e. > 1 kDa, and classes 3-7, LMW-DOC.
259
260 2.4 CO_2 efflux measurements during the experiment
261
262 The CO_2 efflux from the soil containers was measured just before each soil solution
263 sampling using the static chamber method with an infrared gas analyser (ADC LCA-2, the
264 ADC Bioscientific Ltd., UK). To close the soil containers, vented caps were used. Before the
265 gas measurements, a large plastic bag was filled with ambient air in the growth chamber to
266 be used as compensation air for ventilation with a constant CO_2 concentration. To avoid a
267 sudden pressure shock in the container, each cap was equipped with a small hole, which was
268 closed only after placing the cap carefully on the container. The contact edge of the cap was

sealed with a soft rubber gasket, and airtightness was ensured by putting a weight on the cap. The airspace in the container, varying between 1.6 and 3.1 dm^3 in volume, was stirred by a battery-operated fan in the cap. The CO_2 concentration (ppm) in the air was recorded by saving 4-6 readings at 10-second intervals, and the gas flux was calculated from the linear change in CO_2 concentration taking place during the closure.

2.5 Soil analyses at the end of the experiment

At the end of the experiment, the fresh volume and the mass of samples in the containers were measured, and the soil was cut vertically into four similar sectors. The mass of the first sector was determined before and after drying it at 105 °C to determine the volumetric water content. Then the dried sector was used to determine the loss on ignition (LOI) at 550 °C. The second sector was cut horizontally into 5 cm thick slices, out of which enchytraeids were extracted by means of the wet funnel method. To verify the absence of enchytraeids in the control samples, the extraction process was carried out for them as well. The third sector was used for analysing K_2SO_4-extractable OC_{ex} and C_{mic}. The fourth sector was saved for potential further analyses.

2.6 Data processing and statistical methods

The experimental setup was based on the assumption that the net release of DOC and CO_2-C on the time scale of the experiment would be constant over time and could thus be described by a linear model. The measured maximum cumulative mass loss via CO_2-C flux was less than 3 % of the initial dry mass (Fig.1). At the beginning of the experiment, the content of LMW-DOC in all soil types decreased (Fig. 1). This could have resulted from strong microbial assimilation after the nutrient flush caused by the preparation of the soil samples for the experiment. Therefore, data from the first two sampling dates were excluded from all statistical analyses.

The quantities of DOC and CO_2-C were standardised by dividing them by the dry mass of the organic material in the sample. To facilitate the use of C storage changes as a measure of release rate, DOC removed from the soil containers in the soil solution samplings was taken into account in the computation by adding the removed quantity to the measured pool. OC_{ex} also included the dissolved fraction, and therefore DOC removed in the soil solution samplings was added to OC_{ex} at the end of the experiment. The accumulated CO_2-C flux was calculated by assuming that the measured instantaneous CO_2-C flux continued at the measured rate until the next measurement. Because soil solution sampling removes some of the biodegradable DOC, it may potentially decrease the observed CO_2-C release. However, the total sampled DOC represented only 1-4 % of the total CO_2-C loss during the incubation, and we therefore assume the soil solution sampling to have had a negligible effect on the CO_2-C release rate.

The incubation set-up forms a hierarchical data structure, where the levels of the hierarchy are i) sampling event, ii) soil container, iii) soil type, and iv) worm treatment. The application of a mixed linear model allows an analysis of such multi-level data set, where two consecutive sampling events from a soil container are not independent (Goldstein 1995). The principles for the calculation of C release rates follow the methodology presented in Laurén et al. (2012) (Eq. 1):

$$Q_{ijkm} = \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_{ij} t_{ijkm} + a_{ijk} + c_{ijk} t_{ijkm} + e_{ijkm} \quad (1)$$

where Q_{ijkm} is the measured DOC or CO_2-C content per mass unit of soil (µg g^{-1} dry weight) in the soil type i, the treatment j (worms, without worms), container k, and the sampling event m. In the fixed part of the model, α_i is the intercept for soil type i, β_j is the intercept for treatment j, and $\alpha\beta_{ij}$ is the interaction. γ_{ij} represents the release rate of the compound (µg g^{-1} d^{-1}), and t_{ijkm}

is the time (days) elapsed from the beginning of the experiment. The random component includes the intercept a_{ijk}, the slope c_{ijk}, and the residual term e_{ijkm}. Residual variance (v_{ij}) varied between the soil types and the worm treatments, and therefore the analyses were conducted with the weights $1/v_{ij}$. Pairwise contrasts were used to test the differences in the mean release rates between the soil types and the worm treatments, separately for each soil type. The release rates were chosen for the contrasting because they are not affected by the initial C pool contents.

The release rate of OC_{ex} and the accumulation rate of C_{mic} were analysed by means of a mixed linear model (Eq. 2):

$$(Q_{ijk} - Q_{Mi})t^{-1} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk} \quad (2)$$

where Q_{ijk} is the measured compound content (OC_{ex} or C_{mic}) at the end of the experiment (µg g^{-1} dry weight) for soil type i, treatment j (worms, without worms), and container k, Q_{Mi} is the mean compound content at the beginning of the experiment (µg g^{-1}) for the soil type i, and t is the duration of the experiment (days). μ is the mean release/accumulation rate, α_i is the deviation of the soil type i from the mean rate, β_j is the deviation of the treatment j from the mean rate, $\alpha\beta_{ij}$ is the interaction term, and e_{ijk} is the residual term. The analysis was conducted with weighted residuals, as in the analysis of Eq. 1. The calculated release/accumulation rate ($\mu + \alpha_i + \beta_j + \alpha\beta_{ij}$) for each soil type and treatment is directly comparable to γ_{ij} in Eq. 1. A positive release rate refers to increasing compound pool. Multiple comparisons with the least significant difference (LSD) method were used to test the differences among the soil types and among the worm treatments, separately for each soil type.

The normality of the data and the homogeneity of the variances were checked graphically (Q-Q plots, scatter plots). Differences in the soil characteristics among the soil types were tested with the Tukey's test. To calculate the correlations between the initial C pools and the soil characteristics and the correlations between the release rates of DOC, OC_{ex}, and CO_2-C, Pearson correlations were used. Differences at the $p<0.05$ level were considered significant. The statistical analyses were performed by means of the SPSS (Version 20).

3. Results

The cumulative release of DOC and CO_2-C were linear with time ($R^2 > 0.87$) after the initial flush, which occurred during the first two sampling events (Fig. 1). In general, soil type affected the C release more than the worm treatment did (Table 3). There was no significant interaction between the soil type and the worm treatment. The highest CO_2-C release rate (γ_{ij} > 100 μg C g^{-1} d^{-1}) was observed in mor and in slightly decomposed *Sphagnum* peat (soil types 1, 2, and 5 in Fig. 2), while the lowest DOC and CO_2-C release rate ($\gamma_{ij} < 4$ μg C g^{-1} d^{-1}) was found in the highly decomposed peat (soil types 4 and 6 in Fig. 2). The OC_{ex} pool decreased ($\gamma_{ij} < 0$ μg C g^{-1} d^{-1}) in all samples during the incubation (Fig. 2).

HMW-DOC included molecular-size classes 1 and 2. The largest molecules (class 1) were present only in peat (soil types 3-6, Fig. 2, Table 2). During the incubation, the change in class 1 was small ($|\gamma_{ij}| < 1$ μg C g^{-1} d^{-1}). Due to the higher release in class 2 ($\gamma_{ij} > 1$ μg C g^{-1} d^{-1}), the proportion of the HMW fraction (classes 1-2) in the DOC pool increased during the incubation in almost all soil types. The release in class 2 represented 62-70 % of the total DOC release; except in the slightly decomposed *Sphagnum* peat (soil type 5, Fig. 2), where the release was slightly higher in the LMW fraction (classes 3-7). It is noteworthy that the release of LMW-DOC was negligible from highly decomposed peat samples (soil types 4 and 6).

Soil type affected how the released C was divided between DOC and CO_2-C. In peat, 5-9 % of the total C release occurred in the form of DOC; except in the slightly decomposed *Sphagnum* peat (soil type 5), where only 2 % was released as DOC (Fig. 2). In mor, the corresponding share was 3-5 %. LMW-DOC comprised ca. 17 % from the total DOC release for mor and peat samples alike. The release rates of DOC correlated positively with those of CO_2-C (r=0.595, p=0.041). The correlation between the release rates of DOC and OC_{ex} was negative (r=-0.659, p=0.028). The initial DOC pool, especially the HMW fraction (classes 1-2), was larger in peat (soil types 3-6) than in mor (soil types 1-2 in Table 2). There was positive correlation between the initial C pool and the soil water content (r=0.855, p=0.030), and between the OC_{ex} pool and the bulk density (r=0.955, p=0.003).

The defaunation before the incubation was successful, since no enchytraeids were found in the control samples. The quantity of worms was the highest in the mor samples and the lowest in the highly decomposed peats (Table 1). In mor, enchytraeids enhanced the release of CO_2-C significantly by 31-43 % and of DOC by 46-77 % (Fig. 2). Enchytraeids also changed the quality of DOC by increasing the release especially in HMW-DOC (size class 2). There was a tendency for a smaller decrease of OC_{ex} in the presence of enchytraeids, but the effect was significant only in soil type 2 (Fig. 2). No effect of the enchytraeids on the C_{mic} pool was found.

4. Discussion

4.1 C release dynamics

It has been well established that the quality of the organic matter controls its decomposition rate (Prescott 2010), but studies on how this reflects to the quality of the released DOC have appeared only recently (Mastný et al. 2018). Our experiment revealed the most striking differences in C dynamics among the studied soil types. The highest DOC release rate, mainly in the high molecular weight HMW fraction, was found in mor and slightly

406 decomposed peat, and the highest rates of mineralisation (CO_2-C production) were measured
407 for mor and slightly decomposed *Sphagnum* peat. The highest release rates were, however,
408 markedly lower than those obtained for temperate deciduous forest floor material by Park et
409 al. (2002). As expected, the release rates of DOC and CO_2-C were the lowest in the highly
410 decomposed peats, reflecting the poor chemical composition of the litter (Tfaily et al. 2013)
411 and its excessively high water content (Johnson and Damman 1993). However, the
412 proportion of DOC from the total C release was higher in peat than in mor, and largest in
413 highly decomposed peat. This supports the conception that old organic materials, with high
414 lignin content, release larger relative amounts of DOC than young organic materials do
415 (Hansson et al. 2010). It seems that in the course of decomposition the DOC quality further
416 changes, since in highly decomposed peat the released DOC consisted of high molecular
417 weight compounds and the formation of LMW-DOC was negligible.
418
419 Aerobic decomposition conditions and incubation temperature representing the summertime
420 conditions facilitated the dominance of CO_2-C in the total C release. It is likely that in lower
421 temperature the proportion of DOC would have been higher (Moore et al. 2008). Soil
422 microbial biomass was clearly higher in the slightly decomposed *Sphagnum* peat (Table 2),
423 which can explain the higher observed CO_2-C release rate than in the other peat samples.
424
425 The enchytraeids were able to reproduce during the experiment, since the population density
426 increased from the inoculated 8000 individuals m^{-2} to ca. 21 000 in highly decomposed
427 Sphagnum peat and to ca. 176 000 in low fertility mor which fits within the population
428 density range in the field (Didden 1993). The enchytraeids considerably increased the DOC
429 and CO_2-C release in mor, and the tendency, although statistically insignificant, was seen in
430 peat samples as well. Also in previous studies, enchytraeids increased the DOC and CO_2-C
431 release (Briones et al. 1998; Cole et al. 2000, 2002; Laurén et al. 2012). We expected worms
432 to enhance C release in slightly decomposed peat too, as microbial biomass in peat material
433 was substantial providing suitable diet for enchytraeids. The smaller effect of worms on the

C release in peat was probably related to the lower population density of enchytraeids in peat than in mor at the end of the incubation. In all the studied soil samples the microbial biomass was within the generally observed range of about 1-2 % of the total soil C (Martikainen and Palojärvi 1990). Although we found no effect of the enchytraeids on the microbial biomass C, the productivity of soil microbes may have differed between the treatments. Hedlund and Augustsson (1995) suggested that depending on the intensity of grazing, enchytraeids can either increase or decrease the microbial biomass.

The observed low LMW-DOC release rate can be related to the missing input of fresh organic matter and root exudates, which are important sources of labile DOC (e.g. Yano et al. 2000; Kiikkilä et al. 2006). On the whole, the content of LMW-DOC in soil solution is typically very low even though the flux through this pool can be high (van Hees et al. 2005). The biodegradation of LMW-DOC has been observed to lead to accumulation of refractory HMW-DOC (Kalbitz et al. 2003b; Hagedorn and Machwitz 2007). The effect of enchytraeids on the DOC release was most evident in the HMW fraction, because the worms probably enhanced degradation of solid organic matter, and also because the formation of LMW-DOC nearly equals to its' biodegradation rate. The mineralisation probably explained the decrease in the extractable organic C pool, indicating a favourable source of C for microbes. The smaller decrease in the extractable pool in the presence of enchytraeids suggests that the worms enabled the microbes to utilise C from the organic matter as well. This may have resulted from microbial activity in the guts and faeces of enchytraeids (Cole et al. 2000).

4.2 Implications to DOC export estimates

Our aim was to quantify the potential net release rates of CO_2-C and of LMW- and HMW-DOC. Such information is useful for developing and parameterising process-based models of decomposition (Neff and Asner 2001; Manzoni and Porporato 2009). As shown by Laurén et al. (2012) and Laine-Kaulio et al. (2014), release rates can be converted into decomposition-

462 model parameters. An advantage of the laboratory experiment was that it enabled us to
463 control the environmental conditions and to simplify the system by excluding some
464 processes and focusing on the remaining ones. However, in ecosystem level considerations,
465 the role of soil-vegetation interactions have to be accounted for. Therefore, an experiment
466 quantifying the links between the fresh C input, root associated microbes and decomposition
467 process would be a future step for continuing the study.
468
469 Finally we present a "numerical discussion" on how our results, combined with existing
470 literature, could reflect to DOC quality and quantity in water courses. In the simple
471 computation (full description in Appendix 1) we applied the release rates for LMW- and
472 HMW-DOC (this study), average properties of head water catchments in Central Finland
473 (Korkalainen et al. 2007), and biodegradation equation with parameters (Kalbitz et al. 2003a)
474 to produce transport time, role of biodegradation in the transport, and the DOC export load to
475 watercourse (Fig. 4). From the released DOC ca. 19 % was degraded during the transport.
476 Peatland contributed to almost half of the DOC export even though it covered only one fifth
477 of the catchment area. HMW-DOC dominated the export, and negligible amount of LMW-
478 DOC reached the watercourse. The computed export load corresponds remarkably well with
479 the measured DOC export from undisturbed catchments (Kortelainen et al. 2006) (Fig 4) also
480 with a wide range of peatland coverage. Repeating the computation with the no worms -
481 parameter set the export load was from 14 to 20 kg ha^{-1} yr^{-1} lower. Our numerical discussion
482 allows concluding that the decomposing materials i.e. mor and peat, and division of the
483 released C into CO_2, LMW-DOC and HMW-DOC, can play an important role in DOC
484 export to water courses, and ultimately in ecosystem C balance. Enchytraeid worms can
485 substantially enhance the DOC leaching from terrestrial ecosystem to watercourse.
486
487 5. Conclusion
488 We conclude that both the decomposing material and the presence of active soil fauna
489 influence the rate of C release and how the released C is divided into CO_2, and dissolved

490 organic compounds in different molecular size. It is possible to upscale the laboratory results
491 realistically into wider, catchment or even regional level. It is reasonable to claim that a
492 functional, active terrestrial soil fauna can extend its influence from soil environment to
493 aquatic systems, and to ecosystem level C balance as well.
494
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496

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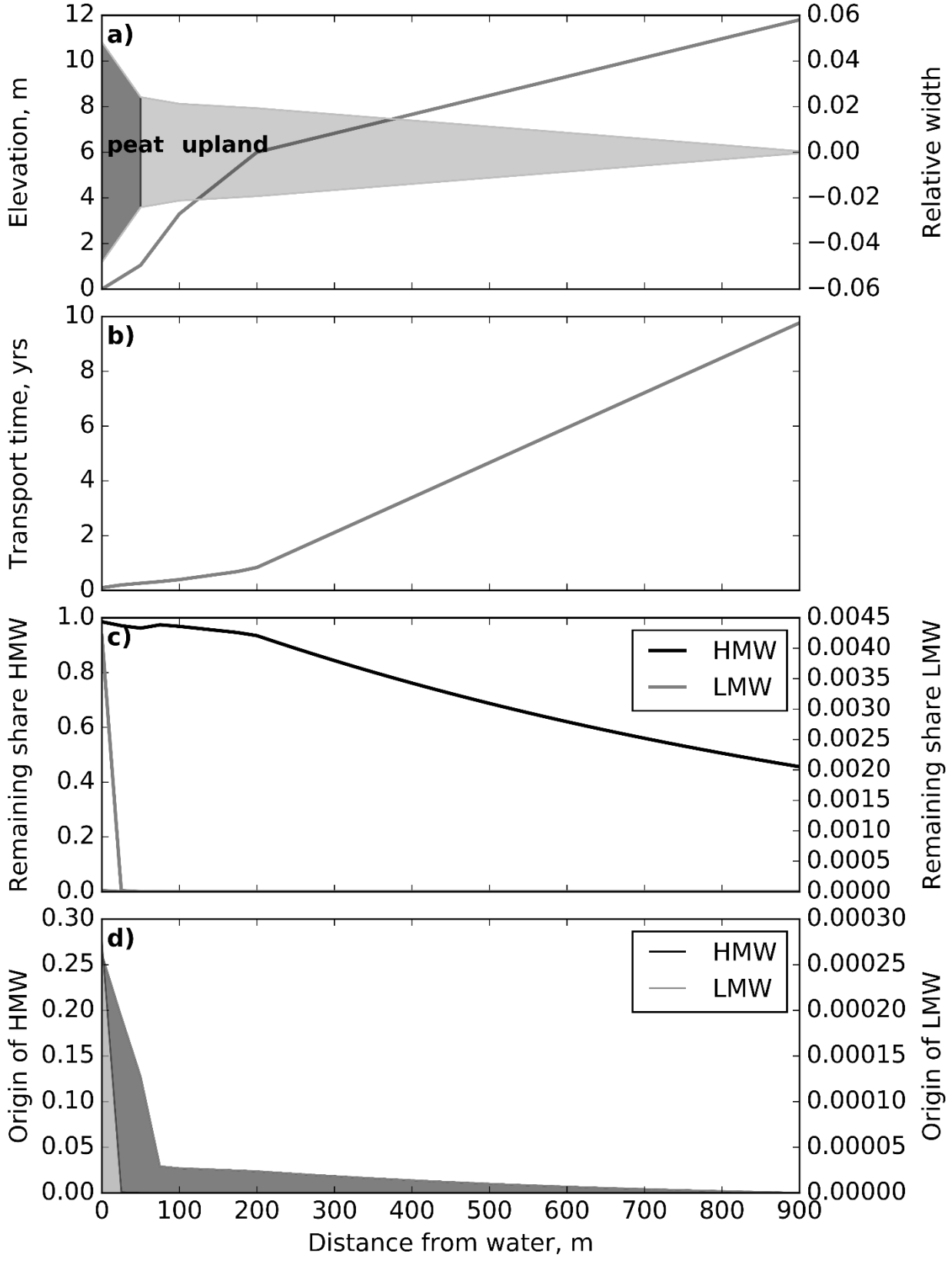
Figure captions

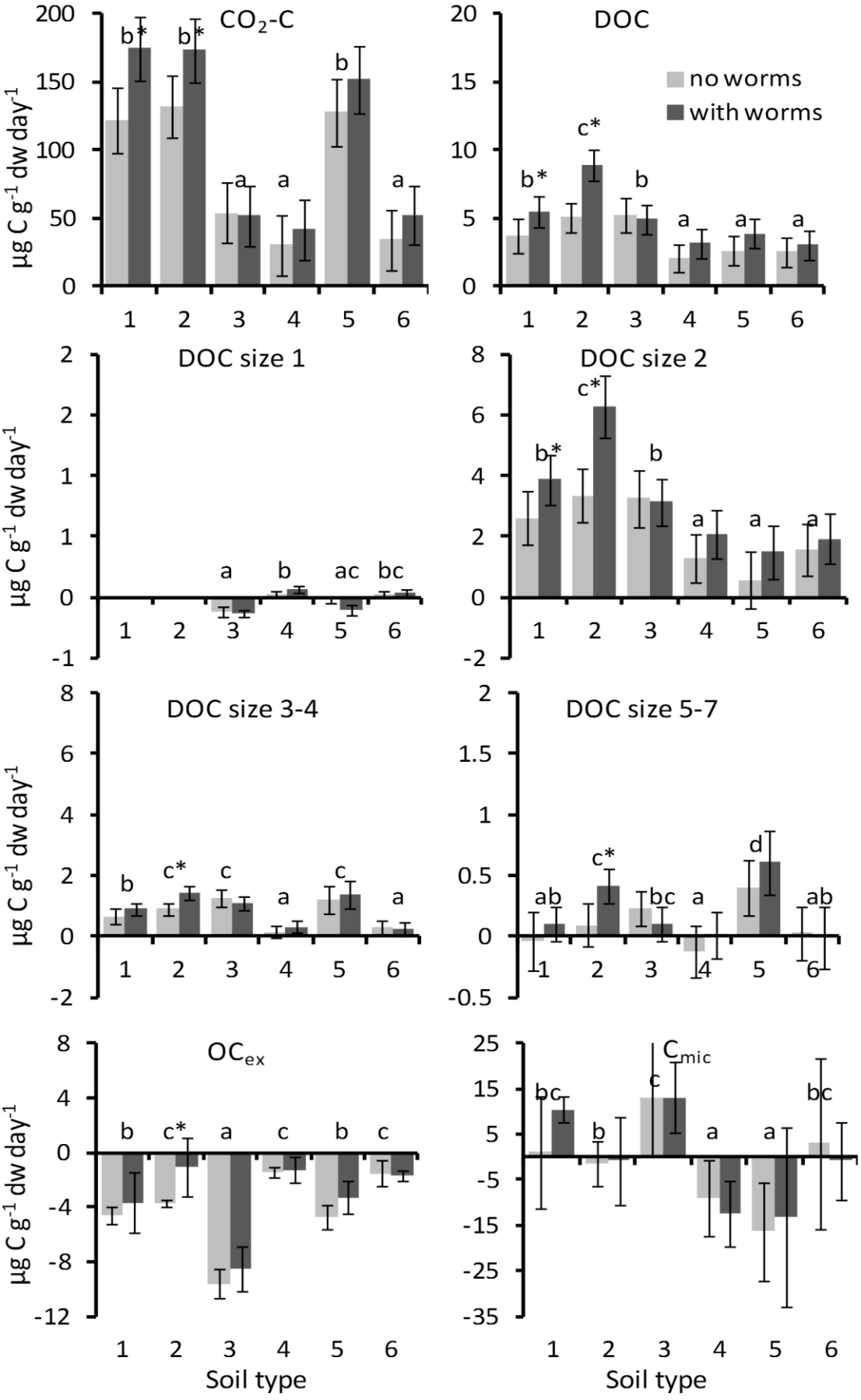
Fig. 1. The mean cumulative CO_2-C release and the mean content of DOC and LMW-DOC in the studied soil types during the incubation as affected by soil type and presence of enchytraeid worms. The error bars indicate one standard deviation. Soil types: 1 = mor, medium fertility type, 2 = mor, low fertility type, 3 = *Carex-Sphagnum* peat, slightly decomposed, 4 = *Carex-Sphagnum* peat, highly decomposed, 5 = *Sphagnum* peat, slightly decomposed, 6 = *Sphagnum* peat, highly decomposed. In two soil solution sampling occasions (the second and the fifth) the ultra-filtration and LMW-DOC analysis was left

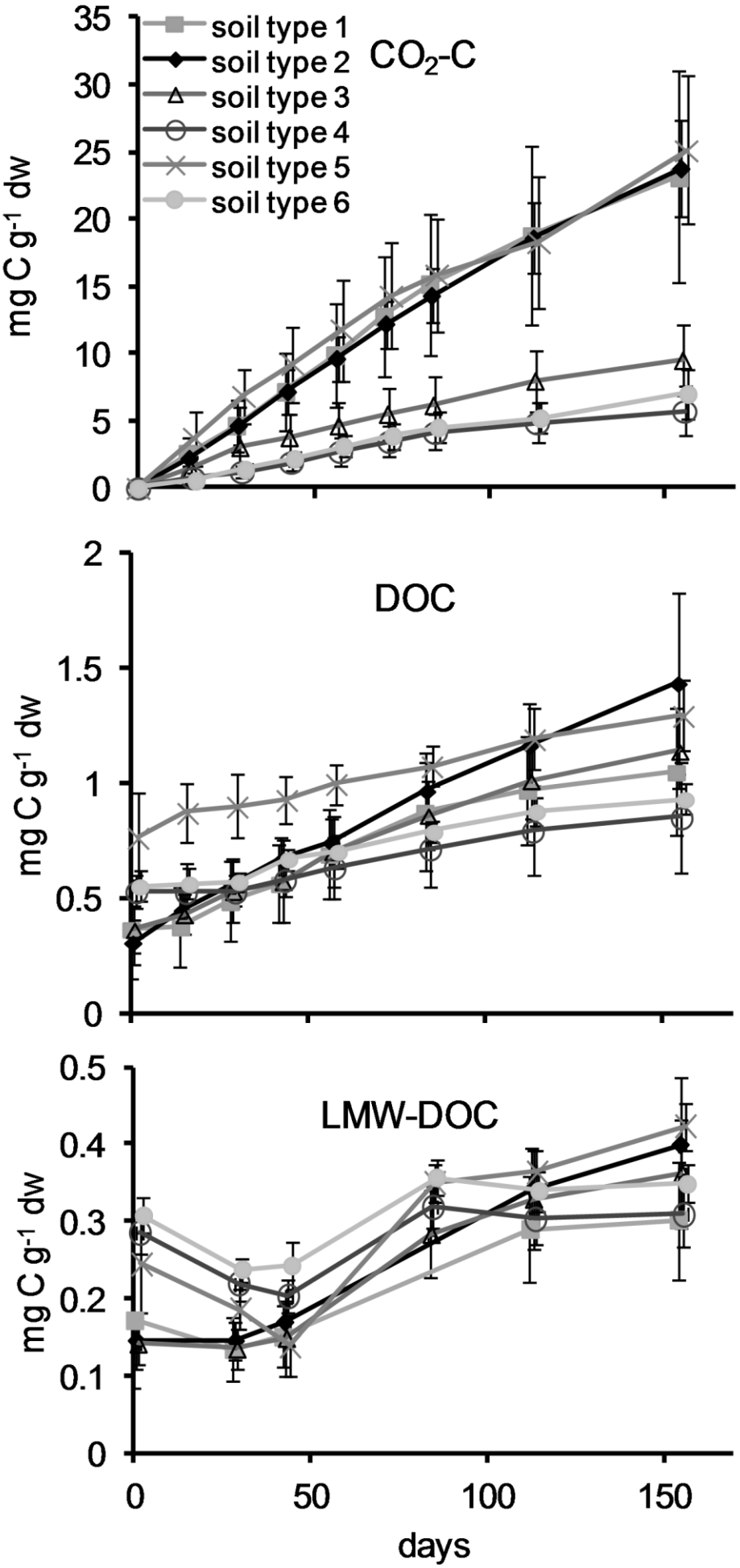
690 outside the analysis procedure to save time and analysis costs. Other missing LMW-DOC
691 observations were connected to damaged ultra-filter membranes.
692
693 **Fig. 2**. The release rates of CO_2-C, DOC, DOC in the relative molecular size classes, and
694 extractable organic C (OC_{ex}), and the accumulation rate of microbial C (C_{mic}). The relative
695 molecular size classes 1 and 2 belong to HMW fraction and the size classes 3-7 to LMW
696 fraction. The thin bars show 95% confidence intervals. When the bar intersects the x-axis,
697 the release rate does not differ from zero at $p<0.05$. Over the columns, the letters denote
698 statistical differences between the soil types at $p<0.05$, and the asterisk (*) shows the
699 significant effect ($p<0.05$) of enchytraeids on the release rate within the soil type. Note the
700 different scales in y-axis. Soil types: 1 = mor, medium fertility type, 2 = mor, low fertility
701 type, 3 = *Carex-Sphagnum* peat, slightly decomposed, 4 = *Carex-Sphagnum* peat, highly
702 decomposed, 5 = *Sphagnum* peat, slightly decomposed, 6 = *Sphagnum* peat, highly
703 decomposed.
704
705 **Fig.** 3. Numerical extension of the incubation results into the DOC export context using
706 literature. Panel a) represents mean flow path characteristics of 782 head water catchments
707 (Korkalainen et al. 2008) as a function of distance to receiving water course. Panel b) shows
708 the mean flow time to water course using the soil information and slope gradient from panel
709 a), and hydraulic conductivity from Koivusalo et al (2008) and Laine-Kaulio (2011). Panel c)
710 combines the flow time information with DOC decay model from Kalbitz et al. (2003a) and
711 illustrates the share of the released LMW-DOC and HMW-DOC that reaches the water course.
712 Panel d) shows the origins and quality of exported DOC. The left Y-axis tells what share of
713 stream HMW-DOC originates from a certain distance (m) from the stream. Similarly, the right
714 Y-axis shows the share of stream LMW-DOC that originates from a certain distance from the
715 stream.
716

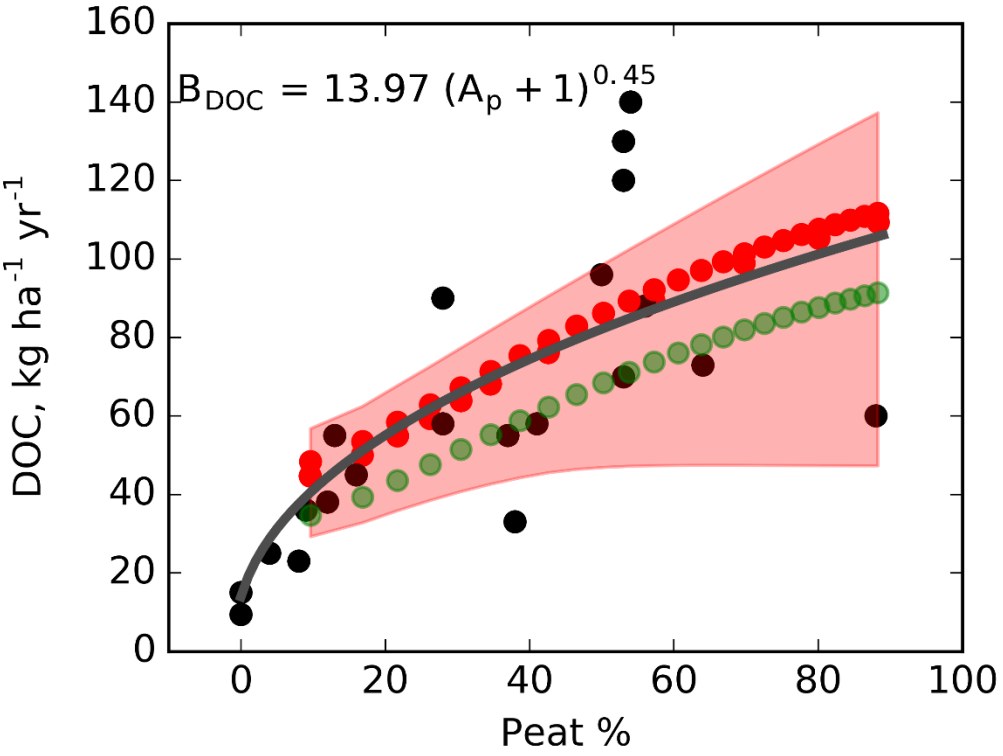
Fig 4. Measured DOC export load after Kortelainen et al. (2006) (black circles), fitting Eq A7 to black circles (gray line), and the export load estimated here (with worms: red circles, without worms: green circles).The red area represents sensitivity of the obtained export to hydraulic conductivity of soil k_{sat} (for the upper limit is applied k_{sat}*10 and for the lower limit k_{sat}*0.1).


a)
peat
upland
Elevation, m
Relative width
b)
Transport time, yrs
c)
HMW
LMW
Remaining share HMW
Remaining share LMW
d)
HMW
LMW
Origin of HMW
Origin of LMW
Distance from water, m

723


CO2-C
DOC
no worms
with worms
DOC size 1
DOC size 2
DOC size 3-4
DOC size 5-7
OCex
Cmic
µg C g-1 dw day-1
Soil type


soil type 1
soil type 2
soil type 3
soil type 4
soil type 5
soil type 6
CO2-C
DOC
LMW-DOC
mg C g-1 dw
days
0
5
10
15
20
25
30
35
0.5
1
1.5
2
0.1
0.2
0.3
0.4
50
100
150


$B_{DOC} = 13.97\ (A_p + 1)^{0.45}$
DOC, kg ha^{-1} yr^{-1}
Peat %
0
20
40
60
80
100
120
140
160

Appendix 1. 'Numerical discussion'

We present a numerical discussion on how our results, combined with existing literature, could reflect to DOC quality and quantity in water courses. The calculation procedure includes the following steps: 1) scaling from soil sample level to site level, 2) scaling from constant to changing temperature, 3) scaling from site to catchment level, 4) computing transport time, biodegradation and DOC export load, 5) results and comparison to literature and 6) evaluation of the computation.

A1. Scaling from soil sample to site level

A catchment consists of upland sites (subscript u) with a mor layer on the top of mineral soil, and peatland sites (subscript p). For upland sites, we set the thickness of mor to 0.04 m according to a typical mor depth in this study. For peatland sites, we assumed that DOC release takes place above the mean water table level, which in was set to 0.31 m according to Ojanen et al. (2010). This peat column sets the frame into which the peat results from this study were embedded: the top part of the peat column (depth 0 - 0.2 m) was parameterized with the results of slightly decomposed peat and the rest (depth 0.2 to 0.31 m) with highly decomposed peat. The parameters for the organic soil layers were derived from this study by averaging (separately with and without enchytraeids) soil types 1 and to 2 to 'mor', soil types 3 and 5 to 'slightly decomposed peat', and soil types 4 and 6 to 'highly decomposed peat' (Table A1). The hectare based dry mass (M_i, kg ha^{-1}) was obtained as a product of the

786 layer thickness and bulk density, and was 35400 kg ha^{-1} for mor , 135600 kg ha^{-1} for slightly
787 decomposed peat and 149600 kg ha^{-1}for highly decomposed peat.
788
789 A2 Scaling from constant to changing temperature
790 The DOC release rates from this study (γ_{ik}, Table A1) were adjusted for temperature using
791 Q_{10} approach (Laurén et al. 2012) and mean monthly air temperature in Finland. The annual
792 release of DOC was obtained by multiplying the adjusted release rate with M_i and summing
793 over the months.
794 $$DOC_{annual\_ik} = \sum_{m=1}^{12} M_i \gamma_{ik} Q_{10}^{\left((T_m - T_{ref})/10\right)} dt_m * 10^{-6}, \quad \text{(Eq A1)}$$
795 where DOC_{annual_ik} is the annual DOC release (kg ha^{-1} yr^{-1}) for soil type i (mor, slightly
796 decomposed peat, highly decomposed peat) and DOC fraction k (LMW, HMW), M_i is the
797 hectare based dry mass of soil type i (kg ha^{-1}), γ_{ik} is the release rate (µg g^{-1} dry mass) of DOC
798 fraction k for soil type i, T_m is monthly mean air temperature (deg C, -9.3, -9.3, -4.8, 1.0, 7.4,
799 12.6, 15.6, 13.4, 8.3, 2.8, -3.2, -7.3, http://ilmatieteenlaitos.fi/kuukausitilastot), dt_m is the
800 length of month m in days, T_{ref} is the reference temperature (15 deg C) and Q_{10} =3.0 is a
801 parameter. Now, the annual DOC release for upland sites (DOC_{annual_uk}) is directly obtained
802 from Eq A1 solved with i = 'mor':
803 $$DOC_{annual\_uk} = DOC_{annual\_mor\_k} \quad \text{(Eq A2)}$$
804 and for peatland sites the release (DOC_{annual_pk}) is obtained as a sum of Eq A1 solved for i =
805 'slightly decomposed peat' and 'highly decomposed peat'.
806 $$DOC_{annual\_pk} = DOC_{annual\_slightly\_decomposed\_peat\_k} +$$
807 $$DOC_{annual\_highly\_decomposed\_peat\_k} \quad \text{(Eq A3)}$$
808
809 A3 Scaling from site to catchment level
810 In this calculation we used average characteristics of water flow paths in head water
811 catchments in Central Finland as analysed by Korkalainen et al. (2007). The authors used
812 782 head water catchments to determine water flow path length, elevation above the

receiving water body, site type (upland, peatland), and relative catchment area as a function of distance to receiving water body (Fig 3a). Peatlands, comprising on average 17% of the area, were located close to water bodies; and uplands had steeper slope than peatlands did. The total length of the characteristic hillslope was 925 m, and for the computation it was discretized into 25 m intervals (dx=25m). The centre points of the intervals are called nodes (number of nodes N = 37). To upscale the DOC release from site to catchment, we located DOC_{annual_uk} to upland nodes and DOC_{annual_pk} to peatland nodes using the soil type information in Fig 3a (node DOC release in node n and fraction k is referred as DOC_{annual_kn}), and the relative area (A_n) for node n. Now the total catchment scale release of DOC was obtained as area weighted average of DOC_{annual_kn} along the flowpath

$$DOC_{tot\_k} = \frac{\sum_{n=1}^{N} DOC_{annual\_kn} A_n}{\sum_{n=1}^{N} A_n}, \qquad \text{(Eq A4)}$$

where DOC_{tot_k} is the total catchment scale release of DOC (kg ha^{-1} yr^{-1}) in fraction k (LMW, HMW), n is the computation node, N is the number of nodes, DOC_{annual_kn} is DOC release in fraction k (kg ha^{-1} yr^{-1}) in node n, and A_n is the relative area of the dx interval where node n is situated.

A4 Transport time, biodegradation and DOC export

Next we assumed that water flow in soil follows the surface gradient described in Fig 3a. We computed the time needed for DOC transport (with water, omitting sorption reactions) from node n to receiving node (n=0 in watercourse) as:

$$t_n = \sum_{m=1}^{n} \frac{dx}{k_{sat\_m}\, g_m \varphi\, 86400} \qquad \text{(Eq A5)}$$

Where t_n is the transport time from a node n through all nodes m between the watercourse and node n (days), dx is discretization interval (25 m), k_{sat_m} is the horizontal saturated hydraulic conductivity and φ is porosity in node m (if peat k_{sat} = 3.4*10^{-4} ms^{-1} and φ = 0.9 Koivusalo et al. 2008; if mineral soil k_{sat}=1.5*10^{-4} ms^{-1} and φ = 0.5 Laine-Kaulio et al. 2011), and g_m is the slope gradient around node m (Fig 3a, m m^{-1}). The transport time is shown in Fig 3b.

Now biodegradation of LMW- and HMW-DOC can be computed using the decay function presented by Kalbitz et al (2003a):

$$DOC_{rem\_kn} = DOC_{annual\_kn} e^{-d_k t_n} \quad , \quad \text{(Eq A6)}$$

where DOC_{rem_kn} is the remaining DOC in fraction k from node n after biodegradation during the transport time t_n (days), DOC_{annual_kn} is the release of DOC in fraction k in node n, d_k is the biodegradation rate constant for fraction k (d_{LMW} = 0.15 day^{-1}, d_{HMW} = 0.0004 day^{-1}, Kalbitz et al. 2003a). Now it is possible to compute the share of the produced DOC that remains nondegradated after the transport (Fig 3c). By scaling DOC_{rem_kn} with the relative area A_n we obtain an estimate of DOC export to water course and its origins from the catchment in LMW and HMW fractions (Fig 3d).

A5 Results and comparison to literature

The total DOC release from peatland was 183.5 kg ha^{-1} yr^{-1} and for upland 42.3 kg ha^{-1} yr^{-1}, thus the area weighted average was 66.1 kg $ha^{-1}yr^{-1}$ for the whole catchment. From this amount 12.6 kg was degraded during the transport and 53.5 kg reached the water course. Peatland contributed to 45.5 % of the DOC export even though it covered only 17 % of the catchment area. HMW-DOC dominated the export, and negligible amount of LMW-DOC reached the watercourse, even though in average 11.9 kg ha^{-1} yr^{-1} LMW-DOC was released at the catchment scale. Therefore, if any LMW DOC is present in watercourse, it has to originate from the close proximity of the water body (<12.5 m).

According to Kortelainen et al. (2006) the range of DOC export in undisturbed catchment is Finland is 10-140 kg ha^{-1} yr^{-1}, and the export increases with increasing proportion of peatland in area. For the same data, Palviainen et al. (2016) presented the following dependency between the DOC export load (DOC_{export}, kg ha^{-1} yr^{-1}) and peatland proportion (A_p %):

$$DOC_{export} = 13.97(A_p+1)^{0.45}. \quad \text{(Eq. A7)}$$

868

869 Plugging in the average peatland proportion of 17% in this example gives 51.3 kg $ha^{-1}yr^{-1}$
870 which is remarkably close to the export load of 53.5 kg $ha^{-1}yr^{-1}$ gained in our simple
871 computation. When the same analysis was computed with the DOC release rates obtained
872 from the incubation without enchytraeid worms (Table A1), the DOC export was 14 kg ha^{-1}
873 yr^{-1} lower.

874

875 To test whether our estimate holds with different shares of catchment peatland area, and
876 different k_{sat} values, we repeated the above computation by extending gradually the peatland
877 coverage from node 1 to node 25 (ref. Fig 3a) giving peatland coverages of 9.4 to 88.3 %.
878 Plotting the computed export loads with the data presented by Kortelainen et al. (2006), and
879 Eq. A7, reveals a remarkably good correspondence (Fig. 4). Repeating the computation with
880 the no worms -parameter set (Table A1) the export load was from 14 to 20 kg ha^{-1} yr^{-1} lower.

881

882 Our numerical discussion allows concluding that the decomposing materials i.e. mor and
883 peat, and division of the released C into CO_2, LMW-DOC and HMW-DOC, can play an
884 important role in DOC export to water courses, and ultimately in ecosystem C balance.
885 Enchytraeid worms can substantially enhance the DOC leaching from terrestrial ecosystem
886 to watercourse.

887

888 A6 Evaluation of the computation

889 The set-up of our computation represents a steady-state situation of DOC fluxes, and the
890 described processes are simplified and many other processes, such as DOC retention in soil,
891 have been omitted. DOC sorption in mineral soil follows a saturating curve as demonstrated
892 by e.g. Kothawala et al. (2008), indicating that DOC is retained efficiently into pristine soil
893 and thereafter gradually the net DOC sorption decreases. It is likely that soil is after 10 000
894 yrs of DOC input in a slower phase of sorption. When interpreted in this context the outcome

is interesting: the magnitudes of DOC release and biodegradation seems plausible even if the role of DOC retention was neglected, suggesting a small net retention of DOC.

The transport mechanism was treated following an equally simplistic way. Implicitly, water is moving in a deep saturated layer with velocity determined by the soil surface gradient, hydraulic conductivity and soil porosity. This transport takes place below the soil frost layer which typically extends to less than 50 cm depth in Finland (Venäläinen et al. 2001); and therefore the transport is mainly unaffected by winter conditions.